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PYRIDOSTIGMINE 2-PAM AND ATROPINE: EFFECTS ON THE
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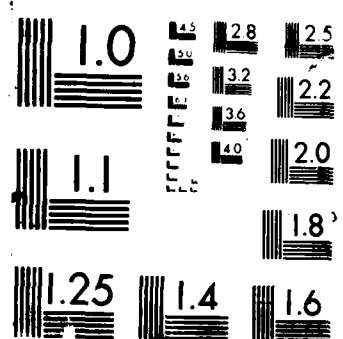
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Pyridostigmine, 2-PAM and atropine: effects on
the ability to work in the heat

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Abstract

Adult (300g), male rats (n=64/group) had access to either tap water or tap water containing 300 mg/L of pyridostigmine bromide (PYR) for 1 week prior to experimentation; this was followed by exercise (9.14m/min, level treadmill) at 30°C to hyperthermic (Tre=42°C) exhaustion. Thirty min prior to the exercise, pyridostigmine (PYR)- and water-drinking groups were treated with an additional intraperitoneally administered regimen: saline (1ml, SAL), atropine sulfate (200ug/kg, ATR), pyridine-2-aldoxime methiodide (50mg/kg, 2-PAM), or atropine plus pyridine-2-aldoxime methiodide (ATR+2-PAM), thus forming eight experimental groups. PYR rats drank significantly more than their tap water drinking counterparts although mean daily food consumption was unaffected by PYR. Mean endurances ranged from 50.4 min (PYR-ATR-2-PAM) to 76.3 min (PYR). The 4 groups receiving 2-PAM manifested a mean endurance of 53.9 min while the 4 groups not receiving 2-PAM had a mean endurance of 66.2 min. PYR-pretreatment elicited a 25% inhibition of plasma cholinesterase while in the two groups consuming PYR and treated with 2-PAM, inhibition was approximately 10%. Blood samples were taken immediately prior and subsequent to exercise in the heat. Hematocrit, total protein, and osmolality were unaffected by PYR and other regimens while osmolality, lactic acid dehydrogenase, creatinine, urea nitrogen, and lactate were elevated by the heat/exercise. While creatine phosphokinase was minimally elevated by the mild exercise, these elevations were exacerbated in the 2-PAM groups. Pyridostigmine consumption for one week reduced circulating cholinesterase activity by 25%. While treatment with 2-PAM restored part of this activity, there were indications (endurance capacity and CPK efflux) that 2-PAM may have subsequent adverse effects on the ability to work in the heat. While indices of heat/exercise injury were ordinarily increased by exercise in the heat, elevations were generally unaffected by pharmacological intervention.

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INTRODUCTION We have recently reported (1,8) that acute administration of large doses of carbamates eliciting significant (40-65%) inhibition of plasma cholinesterase affected endurance and heating rate when rats were exercised in warm environments or sedentarily exposed to hot environments. Alternatively, we have also demonstrated (2) that when pyridostigmine was administered orally in moderate dosages over sub-chronic intervals (7-14 days), then cholinesterase was inhibited by 20-30% and effects on performance and thermoregulation were minimal. Atropine is the prototype anticholinergic drug, and we have reported that, analogous to its sweat suppression effects in humans, atropine significantly inhibited saliva secretion in sedentary rats while its effects on performance have been studied minimally (5,7). The thermoregulatory and performance effects of the cholinesterase reactivator pyridine aldoxime methochloride (2-PAM) have not been investigated. Because of the continuing interest in the potential efficacy of pyridostigmine, atropine, and 2-PAM as prophylaxis and therapy for organophosphate intoxication, we have used a validated model of human heat/exercise injury (3,6) to quantitate the effects of these drugs on thermoregulation and performance.

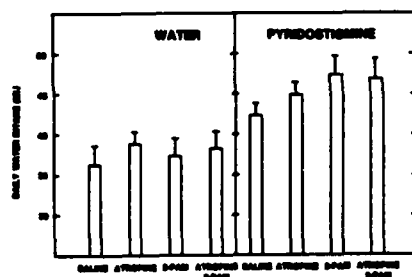
METHODS Groups of adult (300g), male rats ($n=64/\text{group}$) had free access to either tap water or tap water containing 300mg/L of pyridostigmine bromide (PYR) for one week prior to experimentation. During this interval food and fluid consumption were monitored carefully on a daily basis. On the day before an experimental trial permanent indwelling catheters were implanted in the external jugular vein for rapid and convenient blood sampling. Thirty minutes prior to exercise ($9.14\text{m}/\text{min}$, 0° angle of incline) in the heat (30°C) to hyperthermic exhaustion ($T_{re}=42^\circ\text{C}$), both PYR- and water-drinking groups were further treated with one of four additional intraperitoneally administered regimens: saline (1ml, SAL), atropine sulfate (200 $\mu\text{g}/\text{kg}$, ATR), pyridine-aldoxime methiodide (50 mg/kg , 2-PAM), or atropine plus pyridine-2-aldoxime methiodide (ATR+2-PAM). These experimental or control manipulations thus provided a total of 8 treatment groups ($n=16/\text{group}$), 4 of which consumed tap water and 4 of which drank PYR dissolved in their sole source of drinking water.

Small blood samples (0.7ml) were removed immediately prior and subsequent to the heat/exercise contingency. After hematocrits were determined, plasma was separated by centrifugation (4°C , 2000g) and assayed immediately for osmolality and total protein. Aliquots were deep frozen (-20°C) and subsequently assayed for the following variables: cholinesterase, sodium, potassium, lactic acid dehydrogenase, creatinine, urea nitrogen, lactic acid, and creatine phosphokinase (CPK). During the treadmill interval T_{re} and T_{sk} (mid-length, tail skin) were monitored on a minute-by-minute basis. In a few instances runs were terminated voluntarily at 99 min, but usually hyperthermic exhaustion ($T_{re}=42^\circ\text{C}$, animal unable to right itself) occurred much earlier.

Statistical analysis was performed by analysis of variance followed by the application of the Bonferroni T test for multiple comparisons (BMDP Statistical Package, Los Angeles, CA). The null hypothesis was rejected at $p<.05$; a T test for non-paired independent data was performed to determine the effects of pyridostigmine on fluid consumption.

RESULTS AND DISCUSSION Fig. 1 demonstrates that supplementation of the drinking water with pyridostigmine bromide (Mestinon) increased the mean daily fluid consumption. (45.4ml, all PYR groups vs. 37.7ml, non PYR

FIG 1
TAP WATER AND PYRIDOSTIGMINE
CONSUMPTION IN TWO GROUPS OF RATS



groups, $P < .05$). We had previously observed this trend in a separate experiment (2), and attributed this finding to the slightly increased size of the PYR-drinking animals in the previous study. Since Mestigon tablets were dissolved in the drinking water in the current experiments, it is possible that an inert ingredient contributed to this behavior; resolution of this question awaits a trial in which purified pyridostigmine bromide is used.

As can be seen in Fig. 2, PYR consumption-

tion had no adverse effects on endurance in the heat. Our earlier results (2) also confirmed that this regimen of PYR consumption had no effects on this variable. However, it should be noted that when all groups treated with 2-PAM were compared with all non-treated groups, the 2-PAM treated groups had a significantly ($p < .05$) decremented endurance. This observation was consistent in both the water-drinking and PYR-drinking groups.

Hematocrits were measured in duplicate in whole blood samples taken immediately prior and subsequent to exercise in the heat. The results (Fig. 3) indicate that neither PYR pretreatment nor pharmacological intervention had effects on this variable. The slight, but significant, reductions observed in the post-run samples are probably the result of the initial blood removal as well as a hemodilution which occurs during this acute exercise regimen. While exercise in the heat has no effects on circulating

FIG 2
EFFECTS OF PYRIDOSTIGMINE
PROPYLALIS AND TREATMENT REGIMENS ON
THE ABILITY TO EXERCISE IN THE HEAT

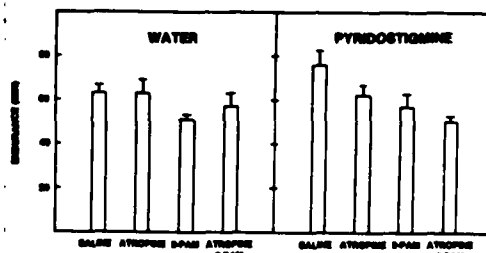


FIG 3
EFFECTS OF PYRIDOSTIGMINE PRETREATMENT
AND ACUTE TREATMENT ON HEMATOCRIT PRIOR AND
SUBSEQUENT TO EXERCISE IN THE HEAT

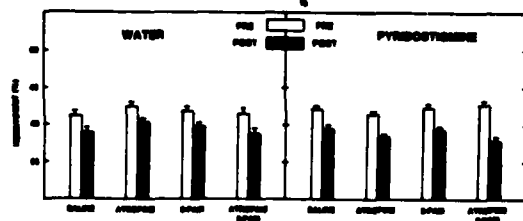
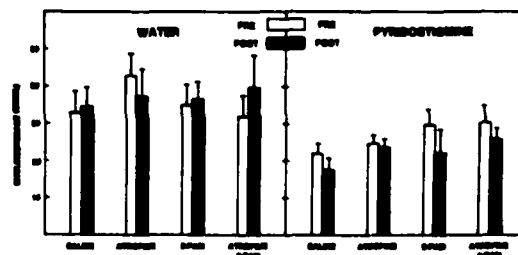


FIG 4
EFFECTS OF PYRIDOSTIGMINE PRETREATMENT
AND TREATMENT REGIMENS ON SERUM
CHOLINESTERASE ACTIVITY



cholinesterase levels, there are two important observations to be noted

from the data illustrated in Fig. 4. Firstly, in the PYR-SAL and PYR-ATR groups PYR consumption for one week ($\bar{X}=13.6$ mg/day) resulted in a significant ($p<.05$) inhibition (~25%) of circulating cholinesterase when compared with the same groups drinking water. Secondly, 2-PAM treatment evidently was effective in partially restoring cholinesterase activity since in the PYR-2-PAM and PYR-ATR-2-PAM groups, cholinesterase levels were 90% of those measured in the 2-PAM and ATR-2-PAM groups.

Fig. 5 demonstrates clearly that exercise in the heat significantly elevated plasma osmolality in all groups while PYR consumption and ATR and/or 2-PAM treatment had no effects on this variable. The data depicted in Fig. 6 are worthy of note and perhaps further investigation. The heat/exercise regimen that is used in the current experiments ordinarily results in a minimal, but significant, elevation in creatine phosphokinase (CPK), and in the SAL and ATR groups, this observation is confirmed. However, the data clearly indicate that the elevations in CPK are exacerbated in the 4 groups treated with 2-PAM. In this, and in several earlier experiments, we have reported increased efflux of intramuscular CPK when endurance has been decremented by experimental intervention. It is unknown presently whether the increased CPK efflux and decreased endurance in the 2-PAM treated groups are related. Interestingly, while not as marked, lactate levels following exercise in the heat (Fig. 7) also appear to be further elevated in the 2-PAM treated groups. Both the CPK and lactate increments in the 2-PAM treated groups are even more noteworthy considering the significantly reduced endurance among these animals. While exercise in the heat elicited significant elevations in several other indices of heat/exercise injury (urea nitrogen, lactic acid dehydrogenase, creatinine, potassium), PYR pretreatment and ATR/2-PAM administration did not affect these variables.

CONCLUSIONS Consumption of 13.6mg PYR per day for 7 days by rats effected a 25% inhibition of plasma cholinesterase which was partially reversed by

FIG 5
EFFECTS OF PYRDOESTERASE CONSUMPTION
AND TREATMENT REGIMENS ON PLASMA OSMOLALITY PRIOR AND
SUBSEQUENT TO EXERCISE IN THE HEAT

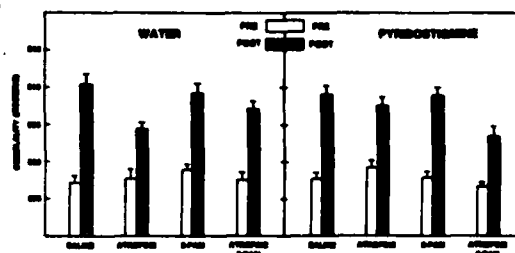


FIG 6
EFFECTS OF PYRDOESTERASE TREATMENT, EXERCISE AND EXERCISE IN THE HEAT
ON PLASMA CREATINE PHOSPHOKINASE LEVELS

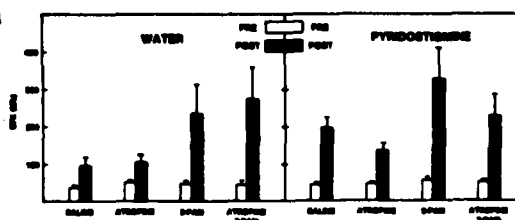
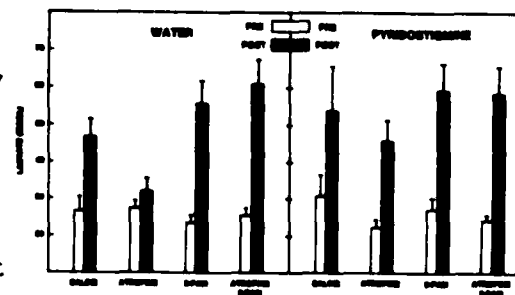


FIG 7
EFFECTS OF PYRDOESTERASE PRETREATMENT AND TREATMENTS ON
CIRCULATING LACTATE LEVELS PRIOR AND SUBSEQUENT TO
EXERCISE IN THE HEAT



2-PAM treatment. All groups of rats treated with 2-PAM manifested significantly decreased endurance capacities, and circulating levels of CPK and lactate were increased despite the reduced endurance. Neither PYR pretreatment nor ATR administration had significant effects on performance or the clinical chemical indices of heat/exercise injury. Effects of PYR prophylaxis appear to be related to the degree of cholinesterase inhibition while the effects of 2-PAM may warrant further investigation.

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